Review

Glial implications in transplantation therapy of spinal cord injury

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Spinal cord injuries are damages that result in complete or partial loss of sensation and/or mobility and affect the life qualities of many patients. Their pathophysiology includes primary and secondary processes, which are related with the activation of astrocytes and microgliacytes and the degeneration of oligodendrocytes. Although transplantation of embryonic stem cells or neural progenitor cells is an attractive strategy for repair of the injured central nervous system (CNS), transplantation of these cells alone for acute spinal cord injuries has not resulted in robust axon regeneration beyond the injury sites. This may be due to the progenitor cells differentiating to the cell types that support axon growth poorly and/or their inability to modify the inhibitory environment of adult CNS after injury. Recent studies indicate that transplantation of glial progenitor cells has exhibited beneficial effects on the recovery and promising future for the therapy strategy of spinal cord injury. In this review, we summarized the data from recent literature regarding glial implications in transplantation therapy of spinal cord injury.

Key words: ***Spinal cord injuries; Neuroglia; Stem cell transplantation***

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Spinal cord injury (SCI), damage of the spinal cord that results in complete or incomplete loss of sensation and/or mobility, is one of the most devastating traumatic conditions that can be encountered by many patients. The pathophysiology of SCI includes the primary damages caused immediately by mechanical forces imparted to the spinal cord and the following secondary damages including processes such as ischemia, inflammation, ionic homeostasis and apoptotic cell death.[1-2] These secondary injury processes also include glutamate-mediated calcineurin activation, bcl-2 antagonist cell death (BAD, a proapoptotic member of the bcl-2 gene family) dephosphorylation,[3] prolonged purinergic receptor activation,[4] exposure of K^+ channels across the demyelinated axonal region[5] and accumulation of intracellular sodium as a result of trauma-induced perturbation of voltage-sensitive sodium channel activity [6]. Recent studies indicated that, in addition to the physiological functions, the activation of microglia cells and astrocytes and the degeneration of oligodendrocytes are important outcomes of the pathophysiology of acute SCI[7] and glial transplantation is a promising therapy for SCI[8-11].

Glial mechanism of SCI

Neuroglial cells, represented mainly by oligodendrocytes, astrocytes and microglia cells, show numerous vital functions in the central nervous system (CNS), such as shaping the micro-architecture of the brain matters, being involved in information transfer and central sensitization, receiving synaptic inputs, releasing gliotransmitters, producing long-range information exchange and acting as pluripotent neural precursors/stem cells.[12-15] Recent studies indicate that clinically-relevant incomplete contusive SCI results in significant loss of axons and glia in the peripheral rim of residual white matter spared by injury[16-17] and that the axonal loss is permanent but the lost glia is largely replaced in

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the residual white matter 6-8 weeks after SCI [17-18], suggesting the involvement of glial cells after SCI.

Decades of studies have pointed out that apoptosis or programmed cell death of oligodendrocytes is one important pathophysiological mechanism of SCI.[19] The pathophysiological apoptosis of oligodendrocytes may be due to the alpha-amino-3-hydroxy-5-methyl-4-osoxazolepropionic acid/kainic acid (AMPA/KA) receptor-mediated toxicity by high concentrations of glutamate following SCI[20] since quantification of AMPA-expressing cells in the white matter of the spinal cord indicates predominant GluR3 expression in oligodendrocytes and a large decline in GluR3-expressing oligodendrocytes after SCI[21]. Recent studies also indicate that N-methyl-D-aspartate (NMDA) receptors are expressed in oligodendrocytes and play some important roles in ischemic diseases.[22] These studies simultaneously indicate that the activities of glutamate receptors of oligodendrocytes are involved in SCI and may be potential therapeutic target for SCI. SCI-induced apoptotic cell death or degeneration of oligodendrocytes will induce myelin degeneration and cause additional disturbances of axonal function.[23] Oligodendrocyte apoptosis and axon demyelination occurring after SCI showed that dynamic changes occurred during the late stage after injury and oligodendrocytes may be replaced. However, through proliferating neuro-glia antigen 2 (NG2) (+) progenitor cells, neural tumor-derived cell line had mixed properties of neurons and glial cells.[24] In addition, apoptosis of oligodendrocytes after SCI may be also associated with apoptosis-1 (apo-1), CD95 (FAS) and p75,[19] c-Jun N-terminal kinase 3 (JNK3) and proteins interacting with the mitotic kinase, nerves in mitosis A I (Pin1)[25] and some oligodendrocyte/myelin-derived axonal inhibitory molecules, such as Nogo-A, myelin-associated glycoprotein (MAG) and oligodendrocyte myelin glycoprotein (OmgP).[26-27]

Increasing evidences have indicated that the activation of astrocytes and microglia cells also contributes to the pathophysiology of SCI. Severely-activated astrocytes may worsen the inflammatory conditions and affect the survival of neurons through secreting excessive proinflammatory cytotoxic cytokines, chemokines and neurotoxic substances, such as the tumor necrosis factor-alpha (TNF-α) that kills oligodendrocytes and the neurotrophin receptor p75 expressed in both neurons and oligodendrocytes after SCI. Astrocytes also undergo changes in the expression of channels and transporters, which could contribute to abnormal excitability and excitotoxicity of neurons.[7, 28, 29] After SCI, water channel aquaporin 4, expressed only by astrocytes, displays early down-regulation at the injury site followed by persistent up-regulation that correlates with increased water content.[30] Another detrimental effect of the formation of glial scar is the up-regulation of chondroitin sulfate proteoglycans (CSPGs), the key components of the extracellular matrix in the nervous system. These molecules, after injury, are secreted by reactive astrocytes and are potent inhibitors for axonal regrowth.[31] However, some studies indicate that, after SCI, reactive astrocytes isolate intact tissues from the lesion, control the concentration of ions, neurotransmitters, neurotrophic factors and metabolic products in the extracellular space, attenuate inflammation, promote the repair of blood-brain barrier and the survival of neurons and oligodendrocytes,[31, 32] suggesting that reactive astrocytes provide essential activities that protect the tissues and preserve the functions after mild or moderate SCI. Similarly, in culture of spinal cord neurons, it shows that astrocytes can mediate the neuroprotective effect of uric acid against glutamate neurotoxicity.[33]

Additionally, proliferation of glial cells also occurs after SCI. After a standardized contusive SCI, cell proliferation including oligodendrocytes, astrocytes, microglia/macrophages, and a high proportion of NG2(+) glial precursors, reaches peak 3 days after injury and continues to be significantly higher than the normal level for many weeks.[34] Some of these dividing cells can differentiate *in vivo* into mature oligodendrocytes or astrocytes,[34] while the number of oligodendrocytes at the impact site is reduced by 93% 7 days after injury but increases three folds 14 days after injury.[35] These results imply one potential role of glial cells in treating SCI.

Implications of glial cells in transplantation therapy of SCI

Most SCIs lead to more or less severe and permanent neurological deficits. Although transplantation of embryonic stem cells or neural progenitor cells is an attractive strategy for repair of the injured spinal cord, transplantation of these cells alone for acute SCI has not resulted in robust axon regeneration beyond the sites of injury. This may be due to the progenitor cells differentiating to the cell types that support axon growth poorly and/or their inability to modify the inhibitory envi-

ronment of adult CNS after injuries. Recent animal studies have shown that glial cells and/or glial progenitor cells, including NG2, Schwann cells (SCs), radial glial cells (RGCs), oligodendrocytes, astrocytes and microglia cells, are present in the normal adult spinal cord and appear to be stimulated to proliferate after SCI,[35-37] holding promise to enhance the functional recovery of injured spinal cord[38-40].

SCs and RGCs SCs are among the most promising candidates for autologous grafting. They can remyelinate the spinal cord lesions after experimental demyelination, leading to functional recovery in some cases of rodent and primate models.[41] However, because the capacity of axonal regeneration is limited in the spinal cord, SCs cannot normally enter the CNS, and the migration of transplanted SCs in the white matter of CNS is inhibited by astrocytes, the functional recovery is minimal. Co-transplantation of SCs with predifferentiated neural stem cells (NSCs) *in vitro* could mildly improve the neural differentiation of NSCs *in vivo* and the injured animals were improved both functionally and structurally, including improved Basso, Beattie, and Bresnahan (BBB) scores, increased axonal regeneration/remyelination, and reduced neuronal loss.[42] SCs may also contribute to neuronal differentiation of NSCs *in vitro* and *in vivo*, thereby, may be helpful for the therapy of SCI. It is found that SCs dissociated and purified simultaneously from the sciatic nerves of 4-day-old rats can significantly increase both the number of survived NSCs dissociated and cloned from the hippocampal tissues of newborn rats and the number of differentiated neuron-like cells that develope axon-like processes, demonstrating that SCs can promote the survival and differentiation of transplanted NSCs in the injured spinal cord.[43] Furthermore, in coculture with SCs, NSCs can differentiate into neurons more readily in the rats with spinal cord contusion injury who have undergone transplantation of NSCs and SCs into the intraspinal cavity, which demonstrates a moderate improvement in motor functions.[44] Therefore, transplantation of NSCs and SCs into the affected area may be a feasible approach for promoting motor recovery in patients with SCI. However, in the study with olfactory ensheathing cells (OECs) and SCs implanted into the intact spinal cord after dorsal column crush (DCC) injury, both OECs and SCs are able to support the axonal re-growth and/or sprout into the lesions.[45]

In the development of the CNS, RGCs are among the first cells derived from neuroepithelial cells. Recent studies have reported that radial glia possesses the properties of NSCs and there is temporal progressive antigen expression in radial glia after contusive SCI in adult rats.[46,47] The acutely-transplanted RGCs can migrate to form bridges across the spinal cord lesions *in vivo* and promote the functional recovery of the injured spinal cord through protecting it against macrophages and secondary damages.[48]

Oligodendrocytes Oligodendrocyte apoptosis is one important pathological mechanism after SCI, while human embryonic stem cell (hESC)-derived oligodendrocyte progenitor cells (OPCs) can express the functional levels of multiple factors with trophic effects on neurons.[8] The neurotrophic activity of hESC-derived OPCs is further demonstrated by their stimulated effects on the neurite outgrowth of sensory neurons of adult rats *in vitro*,[8] which suggests that neurotrophic factors derived from OPCs can impact the survival of axotomized neurons, promote axonal regeneration in interrupted conduction pathways as well as contribute to the functional recovery. In another study, hESC-derived OPCs injected 7 days or 10 months after injury survived, redistributed over short distances, and differentiated into oligodendrocytes. Animals that received OPCs 7 days after injury exhibited enhanced remyelination and substantially-improved locomotor ability. In contrast, when OPCs were transplanted 10 months after injury, there was no enhanced remyelination or locomotor recovery.[49] Furthermore, transplantation of hESC-derived OPCs into the contusive SCI sites in adult rats (200 u) can enhance remyelination and promote the recovery of motor function and a correlation between the presence of demyelinating pathology and the survival and migration rates of the transplanted cells has been noted. However, the transplanted cells in the 50 u injury group survived, exhibited limited migration, but failed to induce remyelination as demyelination in this injury group was absent. The animals receiving a 50 u injury displayed only a transient decline in locomotor function. Importantly, hESC-derived OPCs transplanted into the 50 u injury group did not cause a further decline in locomotion,[50] suggesting that a demyelinating pathology is important as a prerequisite for the function of transplanted myelinogenic cells and that transplantation of hESC-derived OPCs into the injured spinal cord is a promising approach without any harm. These stud-

ies document the feasibility of predifferentiating hESCs into functional OPCs and demonstrate their therapeutic potential *via* the effect of neurotrophic factors at early time points after SCI.

Astrocytes It has been well documented that, in adult rats, astrocytes in the subventricular zone and subgranular layer of the dentate gyrus are NSCs. In either hemi-transectioned or longitudinally-cut spinal cord, there is widespread nestin expression in astrocytes of both the gray and white matters. Culture of the nestin-immunoreactive astrocytes of the lateral cord can generate neurospheres, the cells of which have the ability of self-renewal and are able to differentiate into neurons, astrocytes, or oligodendrocytes.[10] Transplantation of astrocytes derived from embryonic glial-restricted precursors (GRPs) can promote robust growth of over 60% axons to ascend dorsal column into the centers of the lesions with 66% of these axons extending beyond the injury sites and restore the locomotor function after acute transection injuries of spinal cord of adult rats. Grid-walk analysis of GRPs-derived astrocytes (GDAs)-transplanted rats with rubrospinal tract injuries reveals significant improvements in locomotor function. GDA transplantation also induces a striking realignment of injured tissues, suppresses initial scarring and rescues axotomized CNS neurons with cut axons from atrophy. In sharp contrast, undifferentiated GRPs fail to suppress scar formation or support axon growth and locomotor recovery.[51] These results suggest that pre-differentiation of glial precursors into GDAs before transplantation into the injured spinal cord leads to significantly-improved outcomes over precursor cell transplantation, providing both a novel strategy and a highly-effective new cell type for repairing CNS injuries. The contribution of astrocytes to SCI may be related with the remyelination because oligodendrocyte-type 2 astrocyte (O-2A) progenitor cells have been reported to remyelinate the focal areas of demyelinated spinal cord in adult rats and O-2A cells transplanted into the sites of SCI induced with a New York University (NYU) impactor at T_9 of rats could significantly improve the behavior function.[52]

Microglia Additionally, the well-regulated activities of microglia and T cells specific to CNS antigens can contribute to the protection of CNS neural cells and their renewal from adult neural stem/progenitor cells (aNPCs). T cell-based vaccination of mice with a myelin-derived peptide, when combined with transplantation of aNPCs into the cerebrospinal fluid (CSF), can synergistically promote the functional recovery of injured spinal cord. The synergistic effect is correlated with the modulation of the nature and intensity of local T cells, microglial response, expression of brain-derived neurotrophic factors and noggin proteins, and appearance of newly-formed neurons from endogenous precursor-cell pools. These results substantiate the contention that the local immune response plays a crucial role in recruitment of aNPCs to the lesion site and suggest that similar immunological manipulations might also serve as a therapeutic means for controlled migration of stem/progenitor cells to other acutely-injured CNS sites.[53]

Olfactory ensheathing SCs (OESCs) OESCs are unique glial cells, which have the ability to encourage nerve fibres to grow and may be used for treating spinal cord and brachial plexus injuries,[54] while in the study using OESCs and SCs implantation after dorsal column crush (DCC) injury, both OESCs and SCs are able to support axonal re-growth and/or sprout into the lesions. These studies demonstrate the potential therapeutic role of OESCs after SCI. Transplantation of grafts of nasal olfactory mucosa containing OESCs into the partially-removed rat spinal cord can partially recover the movement of hindlimbs and joints. Corticospinal tracing indicates that olfactory mucosa transplantation can restore the severed tract.[55] Clinical studies on transplanting OESCs into patients with SCI have been made in China, Portugal, and other countries and areas.[56] Although there are some positive effects of the transplanted OESCs on axonal regeneration after SCI, the effects and the underlying mechanism of these grafted OESCs on NPCs are not well understood and a number of controversial issues related to OESC biology and transplantation must be addressed to understand the rationale and expectations for OEC cell therapy approaches after SCI.[56] A recent study indicates that the effect of OESCs may be related with the OESCs-derived soluble factors that regulate the proliferation and differentiation of rat NPCs. The conditioned medium from cultured OESCs shows that it can promote the proliferation and inhibit the neuronal differentiation of NPCs.[57] But accumulating anatomical evidences indicate that although axons regenerate within a transplant with OESCs, they do not cross the lesions or reconnect with the neurons on the opposite side to any significant extent and there

are several contradictory reports on the migratory and axon growth-supporting properties of transplanted OESCs.[58,59] It suggests that neuroprotection and promotion of the sprout from the intact fibers may be the main mechanisms contributing to the functional recovery. These findings also reveal the likely limitation of OESCs transplantation for SCI repair and therefore, OESCs transplantation for SCI still has a long way to go.[60]

Some factors should be considered when performing transplantations for SCI, such as time window and transplant site. It showed that a significant increase in cell survival 7 days after SCI was found in the rats receiving rostral and caudal injections of NSCs as compared with the injection directly into the site of injury. A significant increase in cell survival was also found in the rats receiving subacute transplants 9 days after injury as compared with that 28 days after injury.[61] Another problem is the pathways for transplantation. In the majority of reports, cell transplantation is performed by direct local injection with a needle, which might be too invasive for clinical use. Therefore, intravenously delivering neural progenitor cells for the treatment of SCI has been introduced by Fujiwara et al.[62] In their study, neural progenitor cells obtained from E15 fetal hippocampus of transgenic rats expressing green fluorescent protein were transplanted intravenously 24 hours after contusion injury and were found to migrate to the lesion site widely and to demonstrate nestin at an early phase after transplantation. These neural progenitor cells differentiated into neurons, astrocytes and oligodendrocytes, and survived at least for 56 days. These results indicate that intravenously-injected NSCs can migrate into the spinal cord lesion and preserve their potential as neural progenitor cells and that this procedure is a potential method of delivering cells into the lesion for the treatment of SCI.

Perspectives of glial study in treating SCI

Past decades have witnessed increasing interests in strategies to improve neurologic function after SCI. But due to lack of completely understanding of the pathophysiologic events that occur after acute SCI, especially secondary injuries, no very effective therapy is available for severe SCI yet. Since glial cells play vital roles in both physiological neuron functions and pathophysiologic disorders, including their recently-explored functions in controlling brain microcirculation[63] and secretion of neurotrophic factors[13], we should put more efforts on the studies of the glial mechanism after SCI and exploring the therapeutical strategies of transplantations using glial progenitor cells. A recent study using medium with bone morphogenetic protein 4 (BMP4) and leukemia inhibitory factor to culture fetal spinal cord cells provided a means of obtaining pure populations of human spinal cord-derived astrocytes, which could be utilized for further studies of cell replacement strategies or evaluation of therapeutics *in vitro*.[64] Another study indicated that neutralization of ciliary neurotrophic factor reduced astrocyte-mediated scar formation from transplanted NSCs and promoted the regeneration of corticospinal tract fibers after SCI.[65] Treatment with glial growth factor 2 and fibroblast growth factor 2 could enhance oligodendrogenesis and increase the number of EGFP(neg) NG2(+) cells.[66] These studies show an exciting prospect for treatment of SCI with glial cells.

Due to the complicated mechanism of SCI and the limitary effect of single transplantation, efforts have been made to find effective combination strategies to improve the outcomes after injury. Combination interventions have been performed including implantation of SCs plus neuroprotective agents and growth factors administered in various ways, OECs implantation, chondroitinase addition, and elevation of cyclic adenosine monophosphoric acid (AMP). The most efficacious strategy in the acute complete transection/SC bridge model is the combination of SCs, OECs, and chondroitinase administration while the most successful combination strategy for a subacute spinal cord contusion injury is transplantation of SCs combining with elevation of cyclic AMP.[67] In addition, bone marrow stromal cells are also shown to be helpful in OEC transplantation.[68] These studies may provide one effective strategy of glial transplantation for treating SCI.

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